Journal of Research in Agriculture

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Anther Culture of Pepper: Morphological Charactersitics of Fruits of Androgenetic Pepper Lines (*Capsicum Annuum* L.)

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ABSTRACT:

The presented study describes the effectiveness of induced androgenesis in in vitro pepper anther culture. The aim of this study was the establishment of effective technology for induction of embryogenesis in pepper anther culture; development of the embryos into plantlets; successful adaptation and acclimatization of plantlets from sterile to greenhouse conditions, and the breeding process of obtained androgenetic pepper lines in the plastic tunnel conditions. From 19 pepper genotypes under investigation, 12 possessed potential for embryo formation in anther culture. After the acclimatization and adaptation of plantlets, seed material from four pepper genotypes were collected: Piran, Kurtovska kapija SR, Zlaten medal SR and Féherözön. From the collected seed material, breeding processes of androgenetic pepper lines was set up in plastic tunnel (from April-October 2007-2010). The pepper genotypes and androgenetic lines as their products differ among themselves in the length of phonological phases, fruit type and fruit utilization. Detailed study for characterization of morphological and production parameters of the fruits was established that indicate to sort out lines with positive characteristics.

Keywords:
Anther culture, embryo induction, fruit parameters, botanic maturation.

Article Citation:
Liljana Koleva Gudeva and Fidanka Trajkova.
Anther Culture of Pepper: Morphological Charactersitics of Fruits of Androgenetic Pepper Lines (*Capsicum Annuum* L.).
Journal of Research in Agriculture (2012) 1(2): 136-145

Dates:
Received: 10 Sep 2012 **Accepted:** 02 Oct 2012 **Published:** 17 Oct 2012

Journal of Research in Agriculture

INTRODUCTION

Pepper is one of the most important cultures with a long breeding tradition, but on the other hand, scientists very often announce decrease of the pepper yield due to the presence of pathogens and pests. Thus, there is a need for creation of new, pepper genotypes, resulting with higher quality and quantity of the yield (Study for biodiversity in the Republic of Macedonia, 2003). Pepper anther culture is well developed and used method in plant biotechnology and plant breeding. The research on pepper androgenesis was intensive by the end of twentieth century, and still current: Dolcet-Sanjuan *et al*., (1997); Dumas de Valux *et al*., (1981); George and Narayanaswamy (1973); Kim *et al*., (2008); Kuo *et al.,* (1973); Lantos *et al*., (2009); Mityko *et al*., (1995); Mityko and Fari (1997); Özkum and Tipirdamaz (2002) ; Rodeva *et al*., (2004, 2006); Wang *et al.,* (1973). Establishing the effective method of anther culture is an advance in abounding and improving the genetic resources of pepper, Koleva-Gudeva *et al*., (2007, 2009). Using the method of pepper anther culture, fertile androgenetic plants from the genotypes of Kurtovska kapija, Zlaten medal, Piran and Féherözön are created, and also the comparative study was set up for the characterization of the androgenetic lines of pepper.

MATERIALS AND METHODS

Anther-donor plant material and anther culture conditions

Nineteen pepper genotypes were used as anther-donor plants (Table 1). Anther-donor plants were grown under greenhouse conditions. The flower buds were harvested when the corolla was of the same length as the calyx or slightly longer (Figure 1a). The developmental stage of the microspores was determined in microscopic slides of acetocarmine squashes. Flower buds were surface sterilized in 70% ethanol for several seconds, then in 5% calcium hypochlorite $Ca(ClO)_2$ + 2-3 drops Tween 20 for 10 minutes, and rinsed three times in sterile distilled water. After the removal of the filaments, anthers from three flower buds were placed in Petri dish (6 cm), with the concave face down, touching the culture medium. The method of Dumas de Valux *et al*., (1981) was used for induction of embryogenesis.

Table 1. Embryo induction from anthers of different pepper genotypes

Pepper genotype	Total nr. of anthers	Embryogenic anthers (%)	Embryos per 100 anthers	Embryogenic response
Féherözön	1502	17.39 a	32.60 bc	Excellent
Tura	300	17.05 a	17.05 ab	Good
Pritavit F1	330	9.23 abc	9.39 abc	Fair
California wonder	151	6.67 abc	5.67 c	Fair
Zlaten medal SR	1031	6.12 abc	8.97 bc	Fair
Majori	330	5.83 abc	6.73 c	Fair
Piran	823	5.03 abc	34.05 ab	Poor
Zlaten medal ŠT	723	4.29 bc	18.57 bc	Poor
Tomato shaped sweet	360	4.17 bc	4.54 c	Poor
Kurtovska kapija BG	620	2.90 bc	50.55 a	Poor
Kurtovska kapija SR	875	2.73 bc	10.20 bc	Poor
Slatko luta	140	2.43 bc	3.33 c	Poor
Feferona	79	0.00 c	0.00 c	No
Vezena luta	83	0.00 c	0.00 c	No
Sivrija	104	0.00 c	0.00 c	No
Rotund	109	0.00 c	0.00 c	No
Kurtovska kapija TU	236	0.00 c	0.00 c	No
Kurtovska kapija MK	122	0.00 c	0.00 c	No
Bonbona	270	0.00 c	0.00 c	No

Mean within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

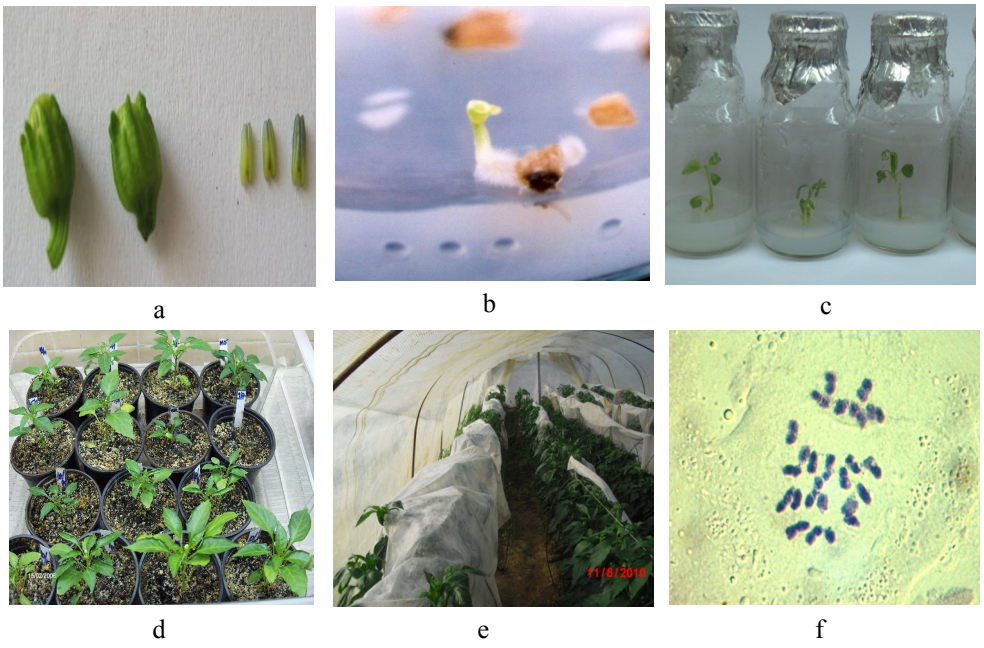

Figure 1. a) Morphological characteristics of pepper anther buds when microspores are in uninucleate phase; b, c) Development of the embryos into plantlets on V_3 medium; d Fully developed plants on acclimatization in climate chamber; e) breeding of androgenic pepper lines in plastic tunnel conditions; f) Caryotype of root tip meristem cells of dihaploid plant obtained via androgenesis 2n=24 (x 1250).

The anthers were cultivated on CP medium + 0.01 mg L^{-1} KIN + 0.01 mg L^{-1} 2.4-D with incubation of eight days in darkness at 35±2ºC, the following four days the anthers were transferred to clime chamber at 25±2ºC with photoperiod of 12h light/12h dark. Afterwards, the anthers were subcultured on R_1 medium + 0.01 mg L^{-1} KIN and placed in clime chamber at 25±2ºC with photoperiodic 12h light/12h dark. Young shoots emerging from the anthers were transferred onto hormone free V_3 media for rooting. Plantlets were planted on sterile mixture of perlite : peat : sand (1:1:1) and acclimatized in clime chamber, and afterwards placed in greenhouse under cover in order to prevent crosspollination (Figure 1 b, c, d).

Field conditions

Different androgenetic pepper lines from the genotypes Kurtovska kapija SR, Zlaten medal SR, Piran and Féherözön, obtained by the regeneration of embryos, using the method of anther culture, were fertile and produce seed material. Seeds collected from the fertile androgenetic plants were used for their characterization in the plastic tunnels in 2007. The collected seeds from the peppers cultivated in 2007, 2008 and 2009 were used for characterization in the experimental year 2008, 2009 and 2010, respectively. The same genotypes Kurtovska kapija SR, Zlaten medal SR, Piran and Féherözön were used as control plants (KKk, ZMk, Pk, Fk).

During the four year of the field investigation (from April – October, 2007-2010), a total number of 36 pepper lines from four pepper genotypes Kurtovska kapija SR, Zlaten medal SR, Piran and Féherözön (KK, ZM, P, F) were subject of study. Experiments were performed at the fields of Faculty of Agriculture, Goce Delcev University in Strumica in plastic tunnel (Figure 2, Walter clime diagrams). In blossom stage, plants were covered with agrill to prevent the foreign pollination between different genotypes (Figure 1e).

Estimation of morphological and production characteristic of fruits

Ten pepper fruits in the stage of botanical

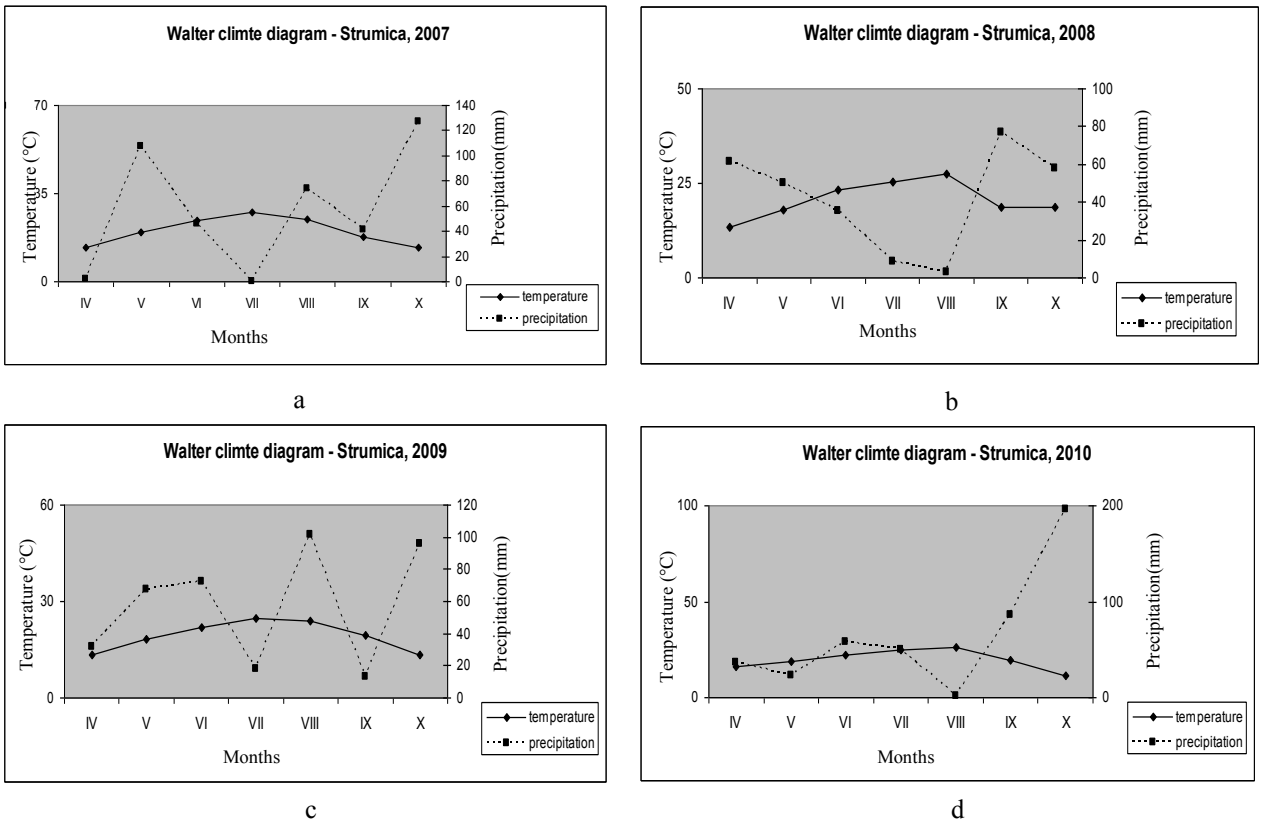

Figure 2. Walter climate diagram for pepper vegetation period in Strumica: a) 2007; b) 2008; c) 2009; d) 2010.

maturation were taken as sampled from each pepper line, and the controls, for analyze of fruit: length, width, total weight, fruit weight without handle and seeds, pericarp thickness, number of fruit locules, weight of dry seed per fruit, number of seeds per fruit, and dry matter according to Ipgri, Avrdc and Catie (1995).

Estimation of number of chromosomes

The number of chromosomes was counted in root tip meristems. The tissue was prepared according to Tjio and Levan (1950) cytological technique, as well as standard "squash" method. The plant material after germination in Petri dishes was pretreated with 8-hydrohyquinoline 0,002 M for 12-24 hours, fixed in aceto- alcohol (1:3), hydrolysed with HCl 1N at 60^{o}C for 9 minutes and stained with 1-1,5% Gomori's hematoxylin by Konstantinov *et al.,* (1985), Figure 1f.

Data analysis

All data on percentage of embryogenic anthers and number of embryos per 100 anthers were subjected to analysis of variance (ANOVA), and the mean values were evaluated at the $p < 0.05$ level of significance using Duncan's Multiple Range Test. Data statistical analyses, concerning morphological and production characteristics of fruits, are done with the software SPSS.10, One-way ANOVA and Duncan posthoc test, with the level of significance 0.05% are carried out.

RESULTS AND DISCUSSION

Not all genotypes under investigation were able to produce embryos (Table 1). After the induction period on CP medium for 12 days the anthers were subcultured on R_1 medium, where since the beginning the embryos showed totipotency, progression in development, growth and shoot formation (Figure 1b). The shoots continued the development on V_3 medium where in absence of plant growth regulators young plants were formed (Figure 1c). The rooting was performed on V_3 medium and well rooted shoots were transferred on sterile

mixture of sand : perlite : peat in the ratio of 1:1:1. In this stage the plants were ready for adaptation and acclimatization (Figure 1d). Seed material from four androgenetic pepper genotypes was collected: Kurtovska kapija SR, Zlaten medal SR, Piran and Féherözön, and were used for breeding process and for investigation of morphological characteristics of fruits in plastic tunnel conditions 2007 – 2010 (Table 2, 3, 4 and 5).

Climate conditions (Figure 2) in the vegetation period, IV - X month, were favorable for the cultivation of pepper fruits. Figure 2 b shows that 2008 was a drought year, but with proper irrigation it did not reflected unfavorably in the cultivation of the studied genotypes of pepper.

The results from the characterization of the androgenetic lines of pepper during 2007 are shown in Table 2. The results showed that line KK3 has significant differences from the control variety Kurtovska kapija for the fruit weight and the fruit weight without handle and seeds. Lines P3 and P4 showed significant differences compared to the control variety Piran for fruit length, total fruit weight, fruit weight without handle, seeds and pericarp thickness. Results for the lines ZM1 and ZM2 compared with the control variety Zlaten medal, showed that line ZM1 does not differ from the control, but the line ZM2 has lower values for the majority of the analyzed characteristics. One of the characteristics for the androgenetic lines obtained from the variety Féherözön is that line F8 did not give fertile fruits as compared to the other lines from the variety Féherözön.

Results from the second experimental year (2008) are shown in the Table 3. The highest value of the fruit width, total fruit weight, fruit weight without handle and seeds, weight of dry seeds and number of seeds per fruit show the line KK1/1 which significantly differ from the results for the other lines and the control. Androgenetic lines obtained from the variety Zlaten medal have significant differences in the value of the weight of dry seeds per fruit, where the seeds from the fruits of line ZM1/3 has the highest value of 1.18 g. Androgenetic line F6/8 has the highest value for the dry

Table 2. Morphological and production characteristics of fruits in different pepper genotypes in botanical maturation grown in plastic tunnel in 2007

Genotype code	Fruit length (cm)	Fruit width (cm)	Total fruit weight g)	Fruit weight without handle and seeds (g)	Pericarp thickness (cm)	Number of fruit locules	Wight of dry seeds/ fruit (g)	Number of seeds/ fruit	Dry matter (%)
KKk	11.50a	4.78a	60.01b	53.73b	0.35a	2.50a	0.49a	66.50a	10.48a
KK1	11.54a	4.77a	67.73ab	60.30ab	0.35a	2.50a	0.39a	54.25a	10.12a
KK3	12.65a	5.12a	86.94a	76.81a	0.38a	2.25a	0.66a	65.75a	9.55a
Pk	12.77b	4.41a	60.23a	52.16a	0.34a	2.20a	0.78a	103.40a	9.96a
P3	10.97b	3.55b	48.22a	38.66b	0.22b	2.50a	0.52a	71.90a	8.62a
P4	16.99a	3.65b	57.40a	47.71ab	0.24b	2.60a	0.98a	103.50a	8.48a
ZMk	12.93a	4.35a	62.26a	52.40a	0.35a	2.20b	0.66a	82.80a	9.96a
ZM1	13.44a	4.30a	62.04a	53.68a	0.32a	2.20b	0.32b	67.80ab	9.62a
ZM2	10.74b	3.18b	31.22b	28.66b	0.26b	3.20a	0.21b	27.00b	8.48b
Fk	7.79b	5.60ab	66.16c	51.84b	0.46ab	4.00a	0.20a	161.80a	5.120b
F5	6.46c	6.35a	84.39b	76.77a	0.50a	3.18bc	0.31a	59.80c	9.491a
F6	9.97a	5.99a	94.24a	85.22a	0.39bc	2.70c	0.47a	91.10b	9.200a
F8	4.76d	4.91b	35.916d	33.80c	0.35c	3.70ab	/	/	9.480a

Mean within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

Table 3. Morphological and production characteristics of fruits in different pepper genotypes in botanical maturation grown in plastic tunnel in 2008

Genotype code	Fruit length (cm)	Fruit width (cm)	Total fruit weight g)	Fruit weight without handle and seeds (g)	Pericarp thickness (cm)	Number of fruit locules	Wight of dry seeds/ fruit (g)	Number of seeds/ fruit	Dry matter (%)
KKk	13.55b	6.50b	89.70c	72.53c	0.38c	2.00c	1.49b	258.80ab	7.30a
KK1/1	14.49ab	7.63a	167.30a	142.90a	0.42bc	2.20c	2.19a	297.60a	7.70a
KK1/8	15.15a	6.59b	138.31b	117.60b	0.42abc	2.40bc	1.45b	198.60b	7.30a
KK3/2	13.87b	6.22b	129.30b	107.80b	0.46ab	3.00a	1.67ab	215.40b	7.20a
KK3/4	14.33ab	6.43b	135.60b	114.95b	0.50a	2.80ab	1.86ab	222.60ab	7.40a
Pк	15.90b	3.58c	46.70c	38.60c	0.28b	3.00a	0.30c	80.80b	8.8ab
P3/3	17.74ab	4.77a	70.00ab	55.73ab	0.42a	2.00b	1.57a	186.80a	7.20b
P3/8	17.70ab	4.58a	74.70a	59.95a	0.24b	2.60ab	1.09b	158.40a	7.80ab
P4/1	18.25ab	3.81bc	58.00bc	48.42b	0.31b	2.40ab	0.53c	61.80b	9.10a
P4/7	20.67a	4.39ab	79.80a	62.83a	0.26b	2.80ab	1.78a	200.40a	8.10ab
ZMk	13.47a	4.99a	88.00a	70.30a	0.39a	2.20a	0.80b	131.80a	6.60a
ZM1/2	14.61a	4.98a	80.99a	66.92a	0.41a	2.40a	0.79b	164.00a	7.20a
ZM1/3	13.30a	5.34a	94.50a	73.53a	0.48a	2.80a	1.18a	190.60a	7.50a
Fk	8.12b	7.18ab	123.50a	98.23a	0.40b	3.80a	1.35a	222.40a	6.40b
F5/2	6.89bc	7.24ab	111.00a	87.85a	0.43ab	3.00b	1.55a	239.80a	5.40d
F5/9	6.59c	7.74a	129.10a	122.50a	0.46ab	3.40ab	1.41a	214.80a	5.60cd
F6/3	10.20a	6.82b	126.20a	104.60a	0.49a	3.20ab	0.71a	114.00a	6.20bc
F6/8	10.55a	6.77b	134.90a	109.90a	0.50a	3.60ab	1.01a	197.20a	7.30a

Mean within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

matter (7.30%) as compared to the other Féherözön lines and the control variety Féherözön.

Results from the third experimental year (2009) are given in Table 4. Characterization of the androgenetic lines from the variety Kurtovska kapija and the control show significant differences only for weight of dry seeds per fruit. The total fruit weight of the control Piran and the weight of the fruit without seeds and handle are the lowest values, as compared to the same parameters of the other androgenetic lines. Regarding results of fruit characteristics of the control and the androgenetic lines of the variety Zlaten medal, there are significant difference for and pericarp thickness.

Results from the fourth experimental year (2010) are given in Table 5. The fruits of line KK3/1 were the longest and with the highest value for fruit weight without seeds and handle as compared to the fruits from the other Kurtovska kapija androgenetic lines and the control. The Piran androgenetic lines and the control significantly differ in the fruit length and the pericarp thickness. The analysis of fruit parameters for Féherözön control and androgenetic lines showed that there is significant difference for the fruit width, total fruit weight, fruit weight without handle and seeds, pericarp thickness, number of fruit locules, weight of dry seeds per fruit and number of seeds per fruit.

Slightest differences in the fruit morphology of androgenetic lines and the mother line are present at variety Zlaten medal SR during the three year investigation period, while the biggest differences are noticed at lines of Féherözön in the first experimental year and lines of Kurtovska kapija in the second experimental year. Although Féherözön genotype showed the highest degree of formation of embryos

Table 4. Morphological and production characteristics of fruits in different pepper genotypes in botanical maturation grown in plastic tunnel in 2009

Genotype code	Fruit length (cm)	Fruit width (cm)	Total fruit weight g)	Fruit weight without handle and seeds (g)	Pericarp thickness (cm)	Number of fruit locules	Wight of dry seeds/ fruit (g)	Number of seeds/ fruit	Dry matter (%)
KKk	13.62a	5.31a	84.78a	71.17a	0.37b	2.00a	0.25b	55.80b	8.50a
KK1/8/1	13.27a	5.71a	77.54a	91.60a	0.402ab	2.00a	0.76a	128.75a	6.00c
KK3/4/5	12.61a	5.56a	95.50a	78.96a	0.44ab	2.40a	0.29b	57.33b	6.50c
KK3/4/3	12.84a	5.29a	83.71a	71.06a	0.44ab	2.20a	0.09b	28.00b	7.50b
Pk	15.08a	3.07a	34.85b	29.31b	0.24b	2.40ab	0.18b	40.0b	6.60a
P3/3/1	15.66a	3.41a	54.04a	43.28a	0.23b	2.00b	0.97b	40.0b	6.00ab
P3/3/3	14.41a	3.62a	49.90a	41.08a	0.34a	2.40ab	0.52b	66.80ab	6.10a
P4/7/3	15.51a	3.47a	51.45a	40.75a	0.26b	2.80a	0.41b	119.40ab	6.50a
P4/7/1	16.29a	3.84a	55.39a	41.62a	0.25b	2.00b	1.12a	138.60a	4.80b
ZMk	14.77a	5.35a	100.61a	73.13a	0.48a	2.60a	0.56a	96.80a	6.10b
ZM1/2/4	15.49a	5.18a	90.46a	75.69a	0.51a	2.60a	0.65a	159.80a	8.10a
ZM1/2/5	13.37a	5.35a	94.30a	92.05a	0.39b	2.60a	0.82a	164.80a	7.60a
Fk	9.67a	7.57a	140.43a	103.32a	0.51a	3.08ab	0.59a	100.20a	5.00a
F6/3/1	10.55a	6.59a	113.82a	90.83ab	0.40b	3.40bc	0.40a	73.60a	5.20a
F6/3/5	10.51a	7.12a	126.27a	97.03ab	0.50a	3.40bc	0.34a	56.60a	5.30a
F5/2/2	7.50b	7.67a	123.10a	92.99ab	0.498a	3.00c	0.85a	143.60a	5.50a
F5/2/3	5.91c	7.30a	109.39a	79.45b	0.514a	4.00a	0.76a	118.80a	5.00a

Mean within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

(32.60 numbers of embryos per 100 anthers, Table 1), the domestic varieties Kurtovska kapija SR, Zlaten medal SR and Piran showed priority in the selection process.

There are several factors affecting androgenesis in many species, such as genotypes (Mityko *et al*., 1995; Rodeva *et al*., 2004), growth of donor plants, pre-treatments of anthers (Özkum and Tripirdamaz, 2002; Koleva-Gudeva, 2003; Ashok Kumar *et al*., 2003), composition of medium (Irikova and Rodeva, 2004; Koleva-Gudeva and Spasenoski, 2007; Dolcet-Sanjuan *et al*., 1997) and the source of plant material. The mechanism of cold and heat-shock treatment for induction of somatic embryogenesis has been explored and discussed by many authors (Dolcet-Sanjuan *et al*., 1997; Dumas de Valux *et al*., 1981). The studies on somatic embryogenesis of pepper (*C. annuum* L.) are in the domain of androgensis: George and Narayanaswamy (1973), Dumas de Valux *et al*. (1981), Mityko *et al*., (1995), Dolcet-Sanjuan *et al*., (1997) and Rodeva *et al*., (2004). According to the literature, the heat thermal stress (+35^{o}C) has greater effect than the cold one (+7^{o}C) in the process of stimulation of macrospore division of pepper (Kim *et al*., 2008). These findings are in agreement with the results obtained in the present study.

From all pepper genotypes under investigation, 12 possessed potential for formation of embryos. The hot genotypes Feferona, Vezena luta, Sivrija and Bonbona and the sweet genotypes Rotund, Kurtovska kapija TU and Kurtovska kapija MK did not show androgenetic potential, i.e. in anther culture did not form embryos shoots (Table 1). The experiment showed that the effectiveness of androgenesis process depends on pepper genotype and the conditions for anther culture

Table 5. Morphological and production characteristics of fruits in different pepper genotypes in botanical maturation grown in plastic tunnel in 2010

Genotype code	Fruit length (cm)	Fruit width (cm)	Total fruit weight g)	Fruit weight with-out handle and seeds (g)	Pericarp thickness (cm)	Number of fruit locules	Wight of dry seeds/ fruit (g)	Number of seeds/ fruit	Dry matter (%)
KKk	11.694ab	4.74a	63.99b	51.73b	0.45b	2.22a	0.43a	69.00a	7.80b
KK1/2	10.294c	5.37a	53.72b	43.22b	0.46b	2.23a	0.28a	45.20a	7.00c
KK3/1	12.691a	5.43a	91.92a	75.72a	0.54a	2.40a	0.45a	38.80a	8.70a
KK4/2	11.074bc	5.21a	61.01b	54.15b	0.48ab	2.41a	0.65a	103.80a	7.80b
Pk	14.763b	3.68a	59.62a	50.33a	0.52a	3.00a	0.17b	27.33b	7.60a
P1/3	17.80a	3.48a	59.89a	48.56a	0.41b	2.40a	0.62ab	69.60ab	7.60a
P2/3	15.01b	3.17a	49.77a	40.41a	0.36b	2.61a	0.29b	31.60b	7.60a
P4/3	17.75a	3.34a	56.29a	44.71a	0.36b	2.40a	0.84a	96.80a	8.00a
Fk	7.93b	6.94b	109.91b	80.38bc	0.51c	3.83a	0.94c	144.00c	7.20ab
F1/2	11.40a	7.73ab	119.64b	95.84b	0.57bc	3.00bc	0.94c	231.00b	6.30cd
F2/5	10.40a	5.79c	81.48c	64.17c	0.53c	2.60c	0.56c	97.40c	6.80bc
F3/3	8.47b	8.39a	154.74a	119.80a	0.89a	3.40ab	1.42b	325.20a	7.90a
F4/5	7.72b	7.02b	109.53b	78.80bc	0.64b	3.20ab	0.59c	86.00c	5.90d
ZMk	15.81a	5.45a	96.98a	90.22a	0.44a	2.60a	0.84a	117.80a	5.80b
ZM2/2	14.01a	3.88b	58.58b	46.63b	0.35b	2.67a	0.64b	124.30a	6.20a

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at P<0.05 for all genotypes except ZMk and ZM2/2.

maintenance. The embryogenesis resulted in embryo formation that developed into plantlets which were acclimatized in climate chamber and greenhouse conditions. Mityko and Fari (1997) concluded that bell-shape varieties have the highest androgenic ability, while the rest showed very low or no androgenic activity, which is consistent with our results, where the bellshape varieties Féherözön and California Wonder showed a higher potential for embryogenesis compared to the hot and the sweet ones. The anthers of Féherözön variety showed low callus formation, but the androgenic ability was the highest on the same medium. In general, once the callus was initiated, the induction of somatic embryos did not occur, which is similar with the results reported by Binzel *et al*., (1996). After successful acclimatization of the regenerated plant, seed material from four pepper genotypes was collected: Kurtovska kapija SR, Zlaten medal SR, Piran and Féherözön, and were used for characterization and breeding process in plastic tunnel conditions 2007 - 2010. The characterization of different androgenic lines compared to the mother genotype showed that there is great variability in some pepper yield-related characteristics from certain lines (Thul *et al.*, 2009). The lines that showed such potential can be used as starting material for future pepper breeding (Portis *et al.*, 2004; Rodeva *et al.*, 2007).

CONCLUSION

From 19 pepper genotypes under investigation, 12 possessed potential for embryo formation. The hot genotypes Feferona, Vezena luta, Sivrija and Bonbona and the sweet genotypes Rotund, Kurtovska kapija TU and Kurtovska kapija MK did not show androgenetic potential. According to the classification of Mityko and

Fari (1997) for identification of androgenetic potential, based on percentage of anthers that give embryos, in our research twelve genotypes showed ability for embryo formation: 1 genotype with excellent androgenetic potential: Féherözön; 1 genotype with good androgenetic potential: Tura; 4 genotypes with fair androgenetic potential: Pritavit F1, Californian wonder, Zlaten medal SR and Majori; 6 genotypes with poor androgenetic potential: Piran, Zlaten medal ŠT, Tomato shaped sweet, Kurtovska kapija BG, Kurtovska kapija SR and Slatko luta; 7 genotypes do not possess androgenetic potential: Feferona, Vezena Luta, Sivrija, Rotund, Kurtovska kapija TU, Kurtovska kapija MK and Bonbona. Collected material will lead to creation of new and improved pepper genotypes, created for specific agroecological conditions. Generally, the further process of selection from all 19 pepper genotypes should be done towards the improvement of domestic genotypes Kurtovska kapija SR, Zlaten medal SR and Piran.

REFERENCES

Binzel ML, Sankhla N, Joshi S, Sankhla D. 1996. Induction of direct somatic embryogenesis and plant regeneration in pepper (*Capsicum annuum* L.). Plant Cell Rep., 15:536-540.

Dolcet-Sanjuan R, Claveira E, Huerta A. 1997. Androgenesis in pepper Effects of carbohydrate and carbon dioxide enrichment. J Amer Soc Hort Sci 122(4): 468-475.

Dumas de Valux R, Chambonnet D, Pochard E. 1981. *In vitro* culture of pepper (*Capsicum annuum* L.) Anthers: high rate plant production from different genotypes by +35ºC treatments. Agronomie 1(10):859-864.

George L and Narayanaswamy S. 1973. Haploid capsicum through experimental androgenesis. Protoplasma 78:467-470.

Ipgri, Avrdc and Catie. 1995. Descriptors for Capsicum (*Capsicum* spp.). International Plant Genetic Resources Institute, Rome, Italy.

Study for biodiversity in the Republic of Macedonia. 2003. First national report, Ministry of environment and physical planning, Skopje 1-217.

Kim M, Jang LC, Kim EJ, Park M, Yoon M. 2008. Embryogenesis and plant regeneration of hot pepper trough isolated microspore culture. Plant Cell Report 27:425-434.

Koleva-Gudeva L, Spasenoski M, Trajkova F. 2007. Somatic embryogenesis in pepper anther culture: The effects of incubation treatments and different media. Scientia Horticulturae 111:114-119.

Koleva-Gudeva L, Trajkova F, Dimeska G, Spasenoski M. 2009. Androgenesis efficiency in anther culture of pepper (*Capsicum annuum* L.). Acta Hort. (ISHS). 830:183-190.

Konstantinov GH, Belcheva RG, Goranov AK. 1985. Rykovodstvo za prakticheski zanaytiy po genetika. Sofia.

Kuo JS, Wang ZZ, Chein NF, Ku SJ, Kung ML, Hsu HC. 1973. Investigation of the anther culture in vitro of *Nicotiana* and *Capsicum annuum* L. Acta Bot. Sin. 15:43-47.

Lantos C, Juhasz GS, Somogyi KO, Vagi P, Mihaly R, Kristof Z, Somogyi N, Pank J. 2009. Improvment of isolated microspore culture (*Capsicum annuum* L.) via co-culture with ovary tissue culture of pepper or maze. Plant Cell Tissue Organ Culture 97:285-293.

Mityko J, Andrasfalvy G, Csillery G, Fari M. 1995. Anther culture response in different genotypes and F_1 hybrids of pepper. Plant Breeding 114:78-80.

Mityko J and Fari M. 1997. Problems and results of doubled haploid plant production in pepper via anther and microspore culture. Acta Hort., 447:281-287.

Özkum D, Tipirdamaz R. 2002. The effects of cold treatment and charcoal on the in vitro androgenesis of pepper (*Capiscum annuum* L.). Turk. J. Bot., 26:131-139.

Rodeva V, Irikova T, Todorova J. 2004. Anther culture of pepper (*Capsicum annuum* L.): Comparative study of effects of the genotype. Biotech and Bitechnol Eq. 18(3):34-38.

Rodeva V, Grozeva S, Todorova V. 2006. *In vitro* answer of Bulgarian pepper *Capiscum annuum* L. genotypes. Genetica (Serbia) 38(2):129-136.

Rodeva V, Koleva-Gudeva L, Grozdeva S, Trajkova F. 2007. Obtaining of haploids in anther culture of pepper *Capsicum annuum* L. and their including in the breeding process. Yearbook 7, Goce Delcev University, Stip, Faculty of Agriculture, 7-17.

Tjio JH, Levan A. 1950. The use of oxuquinoline in chromosomes analysis. Anal. Extac. Exp. Aula Dei, 2(1):21-64

Thul ST, Lal RK, Shasany AK, Darokar MP, Gupta AK, Gupta MM, Verma RK, Khanuja SPS 2009. Estimation of phenotypic divergence in a collection of Capsicum species for yield-related traits. Euphytica 168:189-196.

Portis E, Acquadro A, Comino C, Sergio L. 2004. Effect of farmers' seed selection on genetic variation of a landrace population of pepper (*Capsicum annuum* L.), grown in North-West Italy. Genetic Resources and Crop Evolution 51:581-590.

Wang JJ, Sun CS, Wang CC, Chein NF. 1973. The induction of pollen plantlets *Capsicum annuum* from anther culture. Sci. Sinica 16(1):147-151.